REVIEW ARTICLE

Small Nucleolar RNAs and Pre-rRNA Processing in Plants

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INTRODUCTION

Messenger RNA (mRNA) translation and thereby gene expression depend on the cell's ability to produce vast numbers of ribosomes, the major component of the translational machinery. The complexity inherent in the production of ribosomal RNAs (rRNAs), the key RNA components of the ribosome, is interesting in itself. Add to this the complex nature of the production and regulation of the myriad other components involved in ribosome biogenesis, and this topic becomes fascinating.

Indeed, there has been a recent resurgence of interest in the production of rRNA, the most abundant RNA in the cell, and in the cell biology of the nuclear domain in which ribosome biogenesis occurs—the nucleolus. This interest has been largely stimulated by the discovery of large families of small nucleolar RNAs (snoRNAs) and the analysis of their functions in rRNA production and ribosome biogenesis. Recent advances in resolving the structure of the nucleolus and in the number of protein and RNA probes that are available have also made it possible to relate subcellular structure to molecular events. This is particularly true in plants, in which the superior resolution afforded by the plant nucleolus puts plant science in this area at the forefront of nucleolar structure–function investigations.

In this review, we describe recent studies of rRNA processing, concentrating mainly on the role of snoRNAs. We also compare the different modes of snoRNA production in different eukaryotic kingdoms, highlight the novel organization of snoRNA genes in plants, and present a model that synthesizes our current understanding of the organization of rRNA processing events in the nucleolus.

rDNA TRANSCRIPTION AND rRNA PROCESSING

Virtually all stages of ribosome biogenesis in eukaryotes, including rDNA transcription, precursor rRNA (pre-rRNA) cleav-

age events, methylation, pseudouridylation (i.e., the conversion of some uridines to pseudouridines), and the association of rRNA with ribosomal proteins, occur in the nucleolus (Hadjiolov, 1985; Shaw and Jordan, 1995). The transcription of rDNA generates a pre-rRNA that contains three of the four rRNAs found in ribosomes—the 18S small subunit (SSU), the 25S large subunit (LSU), and the 5.8S rRNAs (Figure 1). The mature rRNAs are produced by processing of the pre-rRNA, which requires a number of snoRNAs and nucleolar proteins (Maxwell and Fournier, 1995; Sollner-Webb et al., 1995; Venema and Tollervey, 1995; Smith and Steitz, 1997; Tollervey and Kiss, 1997).

The pathway and intermediates of pre-rRNA processing have been studied most extensively in yeast (see Lafontaine and Tollervey, 1995; Venema and Tollervey, 1995). Similar processing pathways are followed in other eukaryotes (Sollner-Webb et al., 1995), but the pre-rRNA, intermediates, and mature LSU are larger (the latter up to 28S). Initial cleavage of the pre-rRNA occurs in the 5' external transcribed
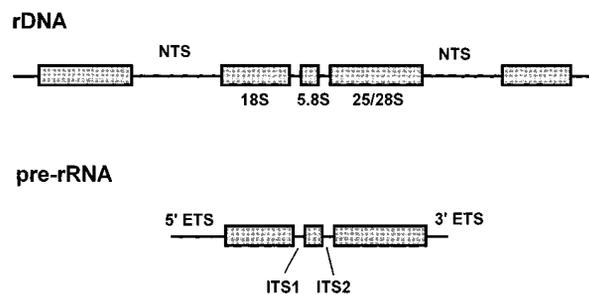
Figure 1. Structure of Eukaryotic rDNA and Pre-rRNA.

(Top) rRNA genes are arranged in tandem, separated by nontranscribed spacers (NTS).
(Bottom) Pre-rRNA transcripts contain the 18S, 5.8S, and 25/28S rRNAs. These are flanked by either the 5' and 3' external spacers (ETS) or internal transcribed spacers (ITS1 and ITS2). The cleavage reactions that generate the mature rRNAs are described in the text.

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spacer (ETS). Subsequently, cleavages occur at the 5′ end of the SSU and in the internal transcribed spacer 1 (ITS1) to generate the mature SSU rRNA and a precursor containing the 5.8S and LSU rRNAs. Finally, cleavage in ITS2 and the 3′ ETS generate mature 5.8S and LSU rRNAs.

In addition to these cleavage reactions, rRNAs also undergo extensive modifications, the most common of which are 2′-O-ribose methylation and pseudouridylation. The majority of these reactions—there are ∼100 of each in vertebrate rRNAs (Maden, 1990a, 1990b; Ofengand and Bakin, 1997)—probably occur cotranscriptionally on the pre-rRNA transcript. Although the role of ribose methylated bases and pseudouridines remains unexplained, they may be involved in refining the common core rRNA secondary structure in the ribosome (Maden, 1990a, 1990b; Maxwell and Fournier, 1995; Bachellerie and Cavaillé, 1997; Ofengand and Bakin, 1997).

Although plant pre-rRNA processing appears to follow a pathway similar to that of other eukaryotes (Grierson, 1982), only a few major processing sites have been identified (e.g., Toloczyki and Feix, 1986; Vincentz and Flavell, 1989), and detailed analysis of all processing sites has yet to be performed. Moreover, no specific sites of ribose methylation or pseudouridylation have been mapped in plant nuclear rRNA. However, we know from early biochemical studies that in wheat germ rRNA there are seven positions at which two adjacent nucleotides are methylated (Lane, 1965), whereas in vertebrate rRNAs, this situation occurs only once (Maden, 1990a).

STRUCTURE AND FUNCTION OF snoRNAs

SnoRNA Structure

Eukaryotic nuclei contain a large number of snoRNAs. These have been grouped recently into three classes: box C/D, box H/ACA, and MRP snoRNAs (Balakin et al., 1996; Kiss-László et al., 1996; Nicoloso et al., 1996; Ganot et al., 1997a). Box C/D snoRNAs such as U3, U8, U13, U14, and the large number involved in ribose methylation (see below) contain two phylogenetically conserved sequences, box C (UGAUGA) and box D (CUGA) (Maxwell and Fournier, 1995; Kiss-László et al., 1996; Nicoloso et al., 1996). These sequences are located near the 5′ and 3′ termini of the molecule, respectively, and are usually flanked by terminal inverted repeats. The stem–box C/D structure formed by base pairing between the inverted repeats acts as a binding site for a set of proteins that stabilize the snoRNA in the form of a ribonucleoprotein particle (snoRNP) (Caffarelli et al., 1996; Cavaillé and Bachellerie, 1996; Watkins et al., 1996; Xia et al., 1997). Box C/D snoRNAs are also associated with the abundant nucleolar protein fibrillarin, which appears to interact with box C/D–specific snoRNP proteins (Caffarelli et al., 1996; Cavaillé and Bachellerie, 1996; Watkins et al., 1996; Xia et al., 1997).

Members of the second major class of snoRNAs, box H/ACA snoRNAs, are characterized by a generalized consensus structure consisting of two stem–loop structures that form in the 5′ and 3′ domains of the snoRNA. The stem–loops are separated by a single-stranded region that contains the phylogenetically conserved box H sequence (ANANNA) downstream of the 5′ stem–loop, and the sequence ACA, which is located close to the 3′ end of the molecule downstream of the 3′ stem–loop (Balakin et al., 1996; Ganot et al., 1997a). Boxes H and ACA are both required for nucleolar accumulation of the snoRNAs and form a binding site for a set of snoRNP proteins (Balakin et al., 1996; Bousquet-Antonelli et al., 1997; Ganot et al., 1997a). MRP RNA is the only member of the third class of snoRNAs. It functions as a ribonucleoprotein particle, RNase MRP.

Functions of SnoRNAs

The functions of U3, U14, and MRP, which are among the more abundant snoRNAs, have been examined in detail in yeast and vertebrates (Maxwell and Fournier, 1995; Smith and Steitz, 1997; Tollervey and Kiss, 1997). U3 and U14, as well as U17, E3, and possibly U22, are needed for the initial 5′ ETS cleavage and the subsequent cleavages that give rise to the SSU. U8 and MRP are required for cleavages that generate the 5.8S and LSU rRNAs. The requirement for multiple snoRNA species and particular proteins for the various cleavage reactions suggests that processing occurs in multicomponent complexes analogous to spliceosomes (Maxwell and Fournier, 1995; Tollervey and Kiss, 1997).

In addition to processing, snoRNPs are also required for rRNA modification, and the two major classes of snoRNAs, box C/D and box H/ACA snoRNAs, specify the sites of ribose methylation and pseudouridylation, respectively. Moreover, each modification site requires a distinct cognate snoRNA, and the majority of box C/D snoRNAs function as guide RNAs that determine the many sites of ribose methylation in eukaryotic rRNAs. Most contain a 10- to 21-nucleotide sequence that is complementary to a region in the rRNA that is being modified. As shown in Figure 2A, these sequences define positions at which the rRNAs become 2′-O-ribose methylated; specificity is determined by the box D or box D–like sequence adjacent to the complementary region (Bachellerie et al., 1995; Cavaillé et al., 1996; Kiss-László et al., 1996; Nicoloso et al., 1996; Tycowski et al., 1996b; Bachellerie and Cavaillé, 1997).

In box H/ACA snoRNAs, short sequences complementary to rRNA are found in the single-stranded internal loops of either one or both of the 5′ and 3′ domain stem–loop structures (Figures 2C and 2D). These regions, and thereby the box H/ACA snoRNAs, act as guide RNAs to determine the positions at which rRNA pseudouridylation occurs (Bousquet-Antonelli et al., 1997; Ganot et al., 1997a, 1997b; Ni et al., 1997).

A

B

C

D

E
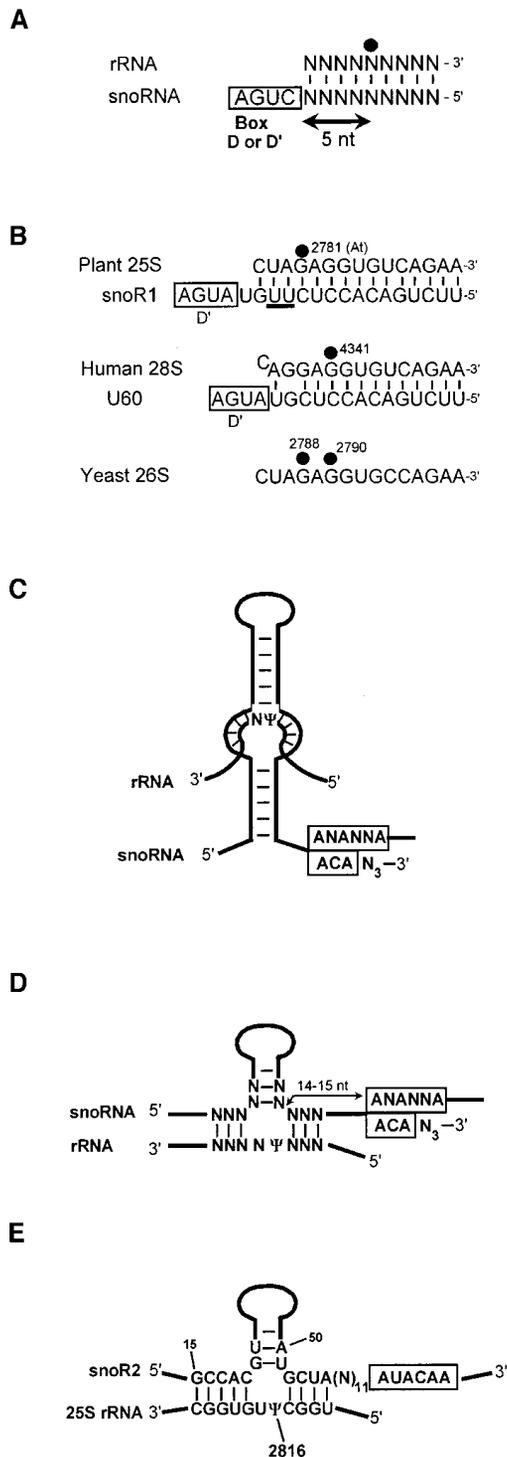
Figure 2. Structure and Function of Box C/D and Box H/ACA SnoRNAs.

(A) and **(B)** Box C/D snoRNAs and the specification of methylation sites.

(A) Model for specification of 2'-O-ribose methylation. The site of ribose methylation, indicated by a filled circle, occurs on the rRNA

Plant SnoRNAs

Although there is no direct evidence for the function of the plant snoRNAs that have been identified to date, their sequence and putative structural similarity to their animal and yeast counterparts point to conserved functions in rRNA processing or modification. The more abundant snoRNAs, U3, U14, and MRP, are all evolutionarily highly conserved. U3 and 7-2/MRP, the first snoRNA genes to be isolated from plants, are transcribed from conventional plant small nuclear RNA (snRNA) promoter elements defined initially for spliceosomal snRNAs (Figure 3A; Solymosy and Pollàk, 1993). However, a major difference between plants and animals is that U3 is transcribed by RNA polymerase III in plants but by RNA polymerase II in animals (Kiss et al., 1991). Plant U14s contain two highly conserved regions that are required for SSU cleavage reactions and a ribose methylation in animals and yeast (Leader et al., 1994). Plant U14s also contain plant-specific regions of unknown function, although one such region corresponds positionally to an essential region, the Y-domain, in yeast U14 (Samarsky et al., 1996). Recently, we have shown that in a fern, this region of U14 differs from that of other eukaryotes, including angiosperms, in that it is considerably larger in size and has the potential to form extensive stem–loop structures (Leader et al., 1998).

In addition to U3 and U14, seven different plant box C/D snoRNA genes and a single box H/ACA snoRNA gene have

within the base-paired region five nucleotides upstream of box D or D' (Kiss-László et al., 1996).

(B) Comparison of complementary regions among plant, human, and yeast 25 to 28S rRNAs, maize snoR1, and the human snoRNA U60. The putative methylation site specified by snoR1 (top; filled circle) aligns with one of the two methylation sites in this region of yeast 26S rRNA (bottom; filled circles) but does not coincide with that found in human 28S rRNA (middle) because of the two extra uridines in snoR1 (underlined), which shift the methylation site two positions upstream.

(C) to **(E)** Box H/ACA snoRNAs and their role in pseudouridylation reactions.

(C) Model for specification of pseudouridylation. A generalized consensus structure of the 5' and 3' stem–loops found in box H/ACA snoRNAs showing the pseudouridylation pocket, which may be in either or both of the stem–loops. It is formed by base pairing of the rRNA with internal loop sequences in the snoRNA, relative to the positions of the conserved box H (ANANNA) and box ACA sequences, which fall downstream of the 5' and 3' domain stem–loops, respectively.

(D) Detail of the pseudouridylation loop. The nucleotide to be pseudouridylated is normally the first unpaired rRNA nucleotide in the pseudouridylation pocket and is usually positioned ~14 to 15 nucleotides from the box H or ACA sequence in the 5' or 3' stem–loop, respectively.

(E) Detail of putative pseudouridylation pocket of maize snoR2.

At, *Arabidopsis thaliana*; nt, nucleotides; Ψ, pseudouridine.

A

snoRNA

B

Splicing

Exonucleolytic
trimming

Mature
snoRNA

C
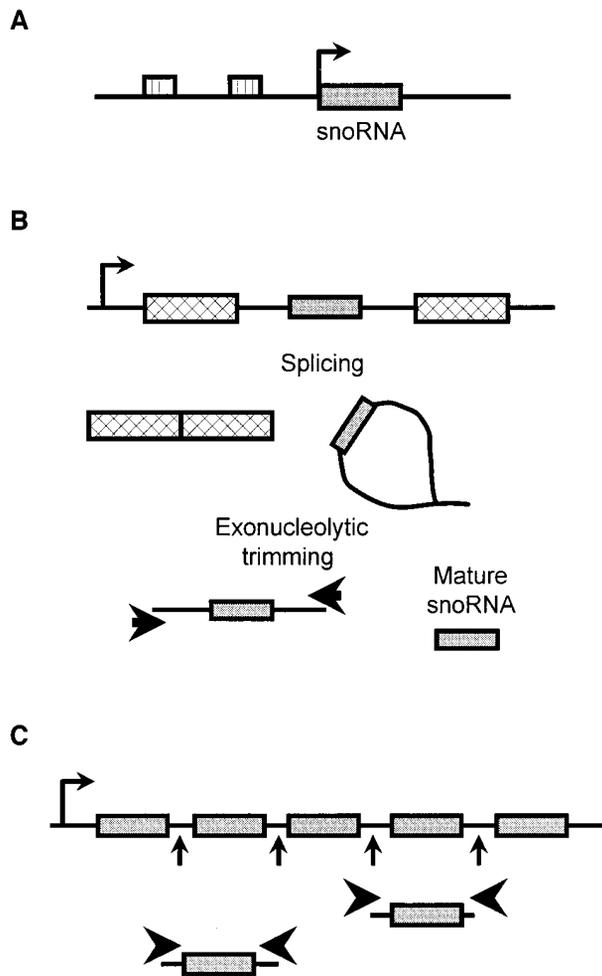
Figure 3. Modes of SnoRNA Expression.

(A) Conventional. SnoRNAs (shaded boxes) that are transcribed (bent arrow) from conventional promoters are characterized by conserved promoter elements (striped boxes).

(B) Intron encoded. (Top) Intron-encoded snoRNAs are transcribed from an upstream promoter as part of a pre-mRNA that includes exons (hatched boxes). (Middle) Splicing releases the snoRNA-containing intron lariat. (Bottom) Debranching linearizes the intron, and exonucleases (arrowheads) remove flanking intron sequences to release the snoRNP.

(C) Polycistronic. Plant snoRNA gene clusters are transcribed (bent arrow) as a polycistron from an upstream promoter and are processed by endonucleases (small arrows) and exonucleases (arrowheads).

been isolated to date (Leader et al., 1997; J.W.S. Brown, unpublished data). Thus, it appears that plants contain families of box C/D and H/ACA snoRNAs whose primary function is to specify sites of ribose methylation and pseudouridylation in rRNAs. All of the box C/D snoRNAs (including U14) contain regions of complementarity to SSU or LSU rRNA that,

consistent with the model describing the specification of 2′-O-methylation (Figure 2A), could determine methylation sites that are conserved in vertebrate and/or yeast rRNAs (Figure 2B; Leader et al., 1997). Similarly, the single box H/ACA snoRNA, snoR2 (Leader et al., 1997), appears to be the analog of yeast snR34 and human U65, which determine pseudouridylation sites in the LSU rRNA. The structure of snoR2 is clearly similar to that proposed for vertebrate and yeast box H/ACA snoRNAs, and regions of complementarity able to form a pseudouridylation pocket are present in this plant snoRNA (Figure 2E).

MODES OF SnoRNA EXPRESSION

Animal and Yeast SnoRNA Genes

Some yeast snoRNAs and the more abundant vertebrate snoRNAs, such as U3, U8, and U13, are expressed from their own promoters (Figure 3A; Maxwell and Fournier, 1995). However, as illustrated in Figure 3B, snoRNAs are most commonly encoded within an intron of a pre-mRNA. This mode of snoRNA expression is utilized by the majority of vertebrate and many yeast snoRNA genes (Kiss and Filipowicz, 1995; Maxwell and Fournier, 1995; Cavaillé and Bachellerie, 1996; Tollervey and Kiss, 1997). Some of the host genes encode proteins that are involved in nucleolus or ribosome assembly, raising the possibility that the coordinated expression of the protein and snoRNAs may be required for ribosome biogenesis (Maxwell and Fournier, 1995). An elegant exception is the vertebrate *UHG* gene in which the snoRNAs U22 to U31 are each encoded within introns of a gene whose final spliced product has no open reading frame (Tycowski et al., 1996a). Apparently, therefore, this gene only exists for the production of these 10 snoRNAs. Processing of intron-encoded snoRNAs is largely splicing dependent. The snoRNA-containing intron is released by splicing, and excess intron sequences are removed by exonucleases (Figure 3B).

Plant SnoRNA Genes

Most plant snoRNAs exhibit a mode of expression that differs from that of the vast majority of animal and yeast snoRNAs. Clusters of snoRNA genes, which occur in either intronic or nonintronic locations, are expressed from their own upstream promoter or as part of a pre-mRNA transcript produced from the promoter of the host protein-coding gene. These features were detected initially in the U14 genes of higher plants, which are clustered and are transcribed polycistronically as precursor snoRNA transcripts (Leader et al., 1994). This gene organization suggests that individual U14s are generated through the action of endonucleases that

cleave between U14 copies and that, as in the intron-encoded vertebrate and yeast snoRNAs, exonucleases remove sequences flanking the snoRNA/snoRNP (Figure 3C; Leader et al., 1997).

More recently, the clustered organization of snoRNA genes has been shown to be widespread in plants. First, other box C/D and box H/ACA snoRNA genes flank the cloned maize U14 genes (Leader et al., 1997). Moreover, polycistronic transcripts containing these snoRNAs are generated from an upstream promoter (Leader et al., 1997), and the release of plant U14s from both intronic and non-intronic constructs demonstrates that processing of the plant snoRNAs is consistent with initial processing by endonucleases (J.W.S. Brown, unpublished data). Second, in Arabidopsis, clustered U14 genes and clusters of four different box C/D snoRNAs, one of which is related to the vertebrate snoRNA U51, have been identified (J.W.S. Brown, unpublished data). In addition, a four-snoRNA gene cluster containing two copies of U51 has been identified in the first intron of the rice *hsc70* gene.

To date, no polycistronic snoRNA gene expression has been found in vertebrates. However, in yeast, the tandem arrangement of two snoRNA genes, snR190 and U14, suggests that their production involves transcription of a dicistron, which is then processed, presumably by an initial endonucleolytic cleavage (Lafontaine and Tollervey, 1995; Tollervey and Kiss, 1997).

NUCLEOLAR STRUCTURE

Ribsomal DNA transcription as well as pre-rRNA processing and modification occur in the nucleolus, and as shown below, these molecular events can be correlated with the structural features therein. The nucleolus is characterized by a lower concentration of chromatin relative to the remainder of the nucleus. Thus, although there are many active gene copies, the nucleolus appears as a "hole" when nuclei are stained with DNA dyes such as 4,6-diamidino-2-phenylindole (DAPI). Nevertheless, on the basis of electron microscopy observations in both animals and plants, several structural features can be distinguished in the nucleolus. These include fibrillar centers—small lightly staining regions; the dense fibrillar component—densely staining material that surrounds the fibrillar centers; and the granular component—an enveloping region of densely packed granules that are generally assumed to be pre-ribosomal particles (Figure 4A; Shaw and Jordan, 1995; Simpson and Filipowicz, 1996).

These structural features are not clearly seen in electron microscopy observations of plant nucleoli, which usually have a more evenly distributed staining pattern. Nevertheless, plant nucleoli offer some decided advantages for detailed ultrastructural studies, particularly those using fluorescence microscopy methods. For example, in many plant cells, the nucleoli are much larger than they are in animal cells, and they tend to have very regular structures (Shaw et al., 1995). This has made it possible to obtain clear images of plant nucleoli that show a great deal of internal structural detail of the localization of various snoRNAs and other components (Beven et al., 1996).

rDNA Transcription and Pre-rRNA Processing

Recently, several groups have used bromo-UTP (BrUTP) incorporation into nascent transcripts, followed by immunofluorescence optical and confocal microscopy, to determine the sites of rDNA transcription in animals (Dundr and Raska, 1993; Hozak et al., 1994) and plants (Figure 4C; Melcak et al., 1996; Thompson et al., 1997). We have shown that in pea roots, the nucleolar transcription sites comprise many small foci (up to several hundred in a large nucleolus) that are distributed evenly throughout the dense fibrillar component (Figure 4C; Thompson et al., 1997). This organization has been confirmed in several dicot and monocot species.

These transcriptional foci coincide with those produced by fluorescence in situ hybridization using probes to the transcribed SSU rDNA and the 5′ ETS, which is present on the nascent transcripts but excised in the first processing steps. However, a probe to the nontranscribed intergenic spacer (Figure 1) does not colocalize (Figure 4C; Shaw et al., 1995; Thompson et al., 1997). This suggests that most if not all of the rRNA transcription foci represent single gene copies that must be packed into domains comparable in size to the resolution limit of this imaging technology (i.e., ∼0.25 μm). In contrast, an antisense probe to ITS1, which is excised in the next stage of pre-rRNA processing, concentrates in the nucleolar regions surrounding the transcription/nascent transcript zones. These regions are broadly coincident with the granular component visualized by electron microscopy (cf. Figures 4A and 4C).

Thus, the nucleolus is organized as a series of concentric layers in which different rRNA transcription and processing steps occur. Initially, individual rDNA genes surrounded by nascent rRNA transcripts (Figure 4B) can be visualized as multiple foci (Figure 4C). As the transcripts move away from the DNA, the early cleavage and processing events occur in a layer that surrounds the transcription sites (Figure 4C). Later processing events, such as ITS1 and ITS2 cleavage and pre-ribosome assembly, take place in an enveloping layer, which is shown in light blue in Figures 4C and 4D.

Localization of Plant SnoRNAs

The localization patterns of plant snoRNAs that are thought to be involved in pre-rRNA cleavage events (i.e., U3, U14, and 7-2/MRP) are consistent with the foregoing layered model for pre-rRNA maturation (Beven et al., 1996). U14 is located in the same pattern of small foci corresponding to the transcription sites and the surrounding layer of nascent
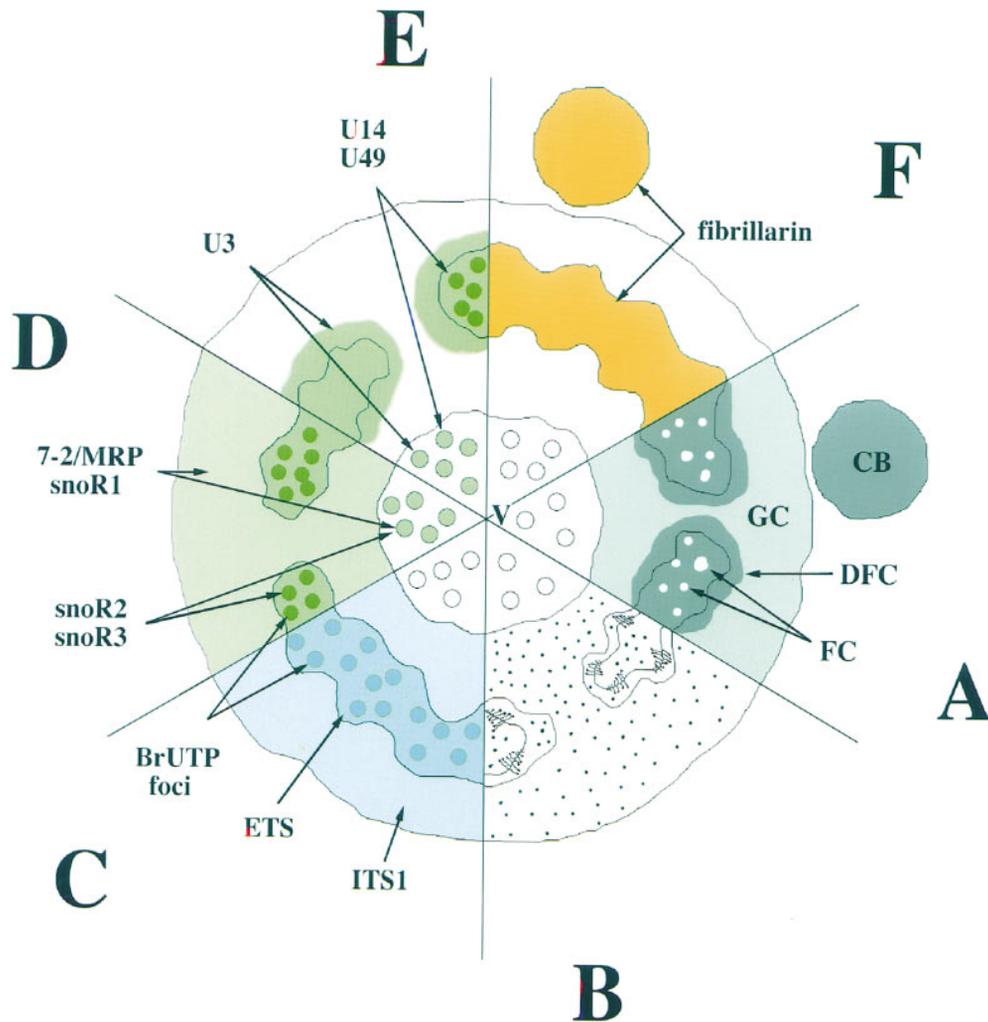
Figure 4. Schematic Diagram of the Observed Localization of Various Components in Plant Nucleoli.

The diagram, which is modified from that presented by Beven et al. (1996), represents the observed structure of a plant nucleolus. The nucleolus is divided into different segments to allow comparison of electron microscopic structure **(A)** with the schematic model of rDNA transcription **(B)** and with the localization of transcriptional foci and rRNA processing events **(C)**, snoRNAs (**[D]** and **[E]**), and fibrillarin **(F)**. In each segment of the diagram, the ETS region, the nucleolar cavity, and the nucleolar periphery are outlined.

(A) Observed electron microscopy thin section ultrastructure. The image shows the dense fibrillar component and coiled bodies (dark gray-green), granular component (light gray-green), and fibrillar centers (white spots in the dense fibrillar component). CB, coiled body; DFC, dense fibrillar component; FC, fibrillar centers; GC, granular component; V, nucleolar vacuole or cavity.

(B) Highly stylized diagram of the possible molecular organization of rDNA transcription complexes. This figure, which is not to scale, places the transcription complexes, drawn here as "Christmas trees" by analogy to Miller spreads (Miller and Beatty, 1969), within the zone defined by ETS probes.

(C) Patterns of BrUTP incorporation and ETS and ITS1 probe labeling. BrUTP is incorporated into many small foci (dark blue dots) within the dense fibrillar component. These foci, most of which probably correspond to single gene copies, are surrounded by a layer of nascent and newly completed transcripts, as indicated by an antisense probe to the ETS region of the pre-rRNA transcripts (medium blue shading). Surrounding this region is a layer in which the ETS has been removed but ITS1 is still present (light blue shading). This region broadly corresponds to the granular component **(A)**.

(D) and **(E)** Labeling patterns of plant snoRNA probes. In **(D)**, 7-2/MRP and snoR1 are shown localized to the granular component (light green shading) and to foci in the nucleolar cavity (light green dots). snoR2 and snoR3 are located at the transcription foci (dark green dots) and in the nucleolar cavity (light green dots). In **(E)**, a U3 probe labels a zone that includes the ETS region but is more diffuse (light green). By contrast, U14 and U49 colocalize with the transcriptional foci within this region (dark green dots). Probes to all three snoRNAs also label foci in the nucleolar cavity (light green dots).

(F) Fibrillarin localization. The nucleolar protein fibrillarin is localized to the dense fibrillar component and to the coiled bodies (orange), in which snoRNAs are barely detectable. Fibrillarin has not been detected in the nucleolar cavity.

transcripts detected by BrUTP incorporation and antisense ETS labeling—a subdomain of the dense fibrillar component (cf. Figures 4C and 4E). U3 is also seen in the same nucleolar region, although its distribution is more diffuse than that of U14 (Figure 4E). This suggests that U3 remains associated with the maturing pre-rRNA longer than does U14, possibly because it is involved in slightly later stages of processing as well as the very early events. 7-2/MRP is distributed quite differently from U3 and U14. It is concentrated in the zones that surround those labeled by the ETS, U3, and U14 probes (Figure 4D). Thus, 7-2/MRP localization correlates well with the ITS1 labeling pattern, which is consistent with a role for 7-2/MRP in the cleavage events that occur in the ITS1 region of the pre-rRNA after excision of the ETS (Beven et al., 1996; cf. Figures 4C and 4D).

A common feature of many plant nucleoli is a central cavity, sometimes called a nucleolar vacuole. We have never detected rRNA or its precursors in this cavity. However, all of the plant snoRNAs we have examined are present to some extent in this region, often as a clear pattern of brightly labeled foci or granules (Figures 4D and 4E; Beven et al., 1996). Thus, this region of the nucleolus may represent a site of storage, pre-processing, or import of snoRNAs. Evidence that the snoRNAs and/or snoRNPs in this region are not mature comes from the localization of the nucleolar protein fibrillarin, which is almost always associated with box C/D snoRNPs; fibrillarin is not seen in the nucleolar cavity (Figure 4F).

Another interesting difference in localization between fibrillarin and its cognate box C/D snoRNAs, such as U3 and U14, is that fibrillarin shows bright immunofluorescence labeling in coiled bodies (Figure 4F), whereas the snoRNAs are barely detectable or entirely undetectable in these structures. Coiled bodies, spherical nuclear structures of unknown function, are up to 1 to 2 μm in diameter and are often associated with the nucleolus. They also contain other nucleolar proteins as well as spliceosomal snRNAs and proteins (Beven et al., 1995; Simpson and Filipowicz, 1996).

Early studies of pre-rRNA methylation suggested that most methylation reactions occur very early and possibly cotranscriptionally on the pre-rRNAs (Maden, 1990a, 1990b). Thus, it could be predicted that the snoRNAs involved in guiding these methylation reactions would associate with the nascent pre-rRNA at the sites of transcription. Indeed, the localizations of most of the novel plant snoRNAs described above also correlate with the model presented in Figure 4 and with their presumed role in methyltransferase or pseudouridylation reactions. For example, two of the plant box C/D snoRNAs (snoR3 and U49) and the box H/ACA snoRNA (snoR2) localize to the nucleolar regions delineated by the ETS antisense probe and by antibodies to fibrillarin, although with some differences in detail, and to the foci in the nucleolar cavity (Figures 4C and 4F). However, a third box C/D snoRNA, snoR1, is unexpectedly localized in a pattern similar to those of 7-2/MRP and ITS1. More detailed analyses of the patterns and dynamics of plant rRNA methylation are needed to explain this distribution.

Finally, the fact that many plant snoRNAs are expressed as polycistrons raises the question of where pre-snoRNA processing to individual snoRNAs occurs. To investigate whether pre-snoRNA expression correlates with nuclear and/or nucleolar structure, we have used probes to the intergenic spacer regions of the polycistronic snoRNA precursors. These preliminary experiments suggest that the intergenic spacers are localized in the nucleolus and, more surprisingly, in the coiled bodies (P.J. Shaw and J.W.S. Brown, unpublished data). Thus, although further detailed analyses are required, it appears that both the nucleolus and coiled bodies may be involved in processing of the plant snoRNA precursors.

CONCLUSION

Making ribosomes efficiently and making efficient ribosomes take an enormous commitment of cellular activity. These fundamentally important processes depend on the appropriate organization of numerous components within the nucleolus and on the many subtle modifications that rRNAs undergo during ribosome assembly. However, our understanding of pre-rRNA processing in plants lags behind that in animals and yeast. As with pre-mRNA processing (i.e., splicing and polyadenylation; Rothnie, 1996; Simpson and Filipowicz, 1996), many components and mechanisms appear to be similar in all eukaryotes, but important and subtle differences in the plant system have been documented. Thus, continued detailed analyses of processing sites and sites of rRNA modification (where indications of differences are already apparent) and of processing components and their functions are required.

Although very few plant snoRNAs have been isolated to date, a major difference from animal and yeast snoRNAs—polycistronic versus intron-encoded expression—has already been identified. However, the possibility remains that plants may also express intron-encoded snoRNAs, and animals may contain polycistronic snoRNAs. Therefore, a better understanding of snoRNA gene organization and expression in all eukaryotic systems is required. The polycistronic clustering of snoRNA genes in plants does, however, raise a number of interesting questions about the plant system. First, what is the nature of the promoters responsible for transcription of the snoRNA gene clusters? Second, how are the processing components (i.e., snoRNAs and proteins) regulated in plants versus other eukaryotes? Third, how and where are the pre-snoRNAs processed and what is the nature of the processing machinery and, in particular, the putative endonuclease(s) that is assumed to generate individual snoRNAs from the polycistronic transcript?

More generally, by taking advantage of the special attributes of plant nucleoli for cell biological analyses, it should be possible to detail the localization of individual (i.e., processed) and polycistronic snoRNAs. Such analyses will

shed light on the distribution and timing of pre-snoRNA processing and the ensuing rRNA modifications as well as on the function of coiled bodies. Moreover, because similar experiments are difficult in animals and yeast, which have much smaller nucleoli, investigations in plants will clearly have an important impact on studies in other kingdoms.

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